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Evidence that Aurora B is implicated in spindle checkpoint signalling independently of error correction

Stefano Santaguida, Claudio Vernieri, Fabrizio Villa, Andrea Ciliberto and Andrea Musacchio

Corresponding author: Andrea Musacchio, European institute of Oncology

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 10 January 2011

Thank you for transferring your manuscript for consideration by the EMBO Journal. As discussed, it has now been seen by three arbitrating referees, who both saw the original referee comments from the previous journal and your detailed rebuttal.

The comments that we received were informal in nature and I summarize the key points below. We will re-review a revised manuscript that includes the experimental data that you offered to add in your rebuttal to the previous journal and that includes the important textural changes suggested by the third arbitrating referee.

Arbitrating referee 1 is concerned about reliance on data with low specificity inhibitors. S/he does not feel the study is sufficiently definitive and does not recommend publication.

referee2 endorses publication. S/he raises the issue of semantics of what is meant by 'auroraB has a direct role in the SAC'. A more careful level of formulation should be used to explain the key finding, namely that Aurora B's checkpoint role can not be explained by its function in error correction, because it is still required for the checkpoint under conditions were microtubules are fully depolymerized, i.e. where error correction can not occur. The same point was noted by referee 2 on the previous version.

The referee requests addition of the experiments outlined in your rebuttal to the previous journal, and notes 'I suspect that in addition to these new data a very clear and careful choice of words and arguments will be needed so that it will be as clear as possible what the Musacchio manuscript shows and what it does not show, and why the authors favor one interpretation over others. As one can see from the long list of previous publications on this topic, and from the comments by these

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Reviewers, this is both a complicated problem and a field which has a long history of controversy and strong opinions.'

referee 3: endorses publication, but requests that the following issues be addressed by rewriting:

> a more critical discussion of Yang et al.:
' the conclusion that Aurora B is not required for spindle checkpoint signaling in the presence of high concentrations of nocodazole is completely dependent on the assumption that 100 nM Hesperidin inhibits all Aurora kinase function in vivo. Secondly while concluding that previous published work was flawed by the fact that nocodazole concentrations used in the previous studies were too low, Yang et al. do not directly test of this conclusion by carrying out side-by-side analyses of mitotic duration in Hesperidin with the same RPE1 cells under varying concentration of nocodazole. Most previous studies were done with Hela cells, which in the Yang et al. study do not seem to show the very strong dependence on drug concentration that the RPE1 cells do.'

> A better discussion of ref 20: 'contrary to the author's claim in the rebuttal, Vanoosthuyse and Hardwick also used chemical depolymerization of microtubules combined with the analog-sensitive aurora mutant to verify their claim for a direct role of Aurora kinase in checkpoint signaling. Indeed, I find the Vanoosthuyse and Hardwick study much more compelling since they use the analog-sensitive Aurora allele to avoid off-target effects of inhibition.'

> 'It is oversimplifying to equate that time to adaptation or duration of mitotic arrest to the measure of checkpoint signal strength. This is because other factors such as the turnover of Cdc20 and Cyclin B play important roles in dictating whether cells remain arrested or exit from mitosis after a drug-induced arrest.

> The Ndc80 discussion should include citation of Genes Dev. 2003 Jan 1;17(1):101-14

In our view, referee 1 should be addressed texturally by carefully describing the complementary approaches used to arrive at what is hopefully a compelling conclusion. Referee 3 has to be addressed by re-writing. The textural changes and experimental data discussed in your rebuttal in response to referee 2 of the previous version should be included in revision.

Thank you for the opportunity to consider your work for publication. I look forward to your revision, which we would have re-reviewed by arbitrating referee 2, as new data will have been added and since it it is our policy to review all data.

Yours sincerely,

Editor
The EMBO Journal

1st Revision - authors' response 21 January 2011

We were in general very pleased with the comments from the arbitrating referees (ARs), with the exception of course of AR1, who did not recommend publication.

Within the section AR2 we also include a list of new experiments and clarifications added to the manuscript to satisfy the concerns of the original reviewers.

<u>AR1</u>

As the main concern of AR1 appears to be the lack of specificity of inhibitors, we can only argue that this is a general problem in biology (e.g. similar concerns affect RNAi-based experiments). We would like to point out that we use a collection of Mps1 and Aurora B inhibitors and their combinations, to a level that is uncommon for any previous publication on Aurora B. In this respect, while we agree in principle with AR1, we point out that we did our best to avoid specificity problems that are the undesired companion of any experiment in biology.

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AR2

We thank AR2 for endorsing publication. To satisfy AR2's concern, we now avoid, throughout the text (including the title), the expression "direct role of Aurora B in the checkpoint", and replace it with the expression "role of Aurora B in the checkpoint independently or error correction". Although we feel that we provide substantial evidence for a direct role of Aurora B in the checkpoint, we also feel that this alternative expression leaves the door open to the possibility that Aurora B facilitates checkpoint function in less direct ways, for instance through a contribution to kinetochore assembly. We note that our data, and additional data provided with this new version of the manuscript, seem to argue against this possibility. Yet, we hope that this new expression settles the issue and can be accepted as a reasonable compromise.

We have also added, as per AR2's request and following the requests of referees 1 and 2 of the original version of the manuscript, several new panels that we believe confirm and in fact reinforce our conclusions. In summary, the list of new data include:

Panel 2A, where we illustrate the response of HeLa cells to growing concentrations of nocodazole at a fixed concentration of hesperadin. This result confirms, as explained in the main text, that 3.3 µM nocodazole is an appropriate concentration to draw our conclusions. Of note, we use a much higher concentration of nocodazole or of a different microtubule depolymerizer in a different figure (Figure S4) and show that the results are essentially indistinguishable relative to what we observe at 3.3 µM nocodazole. Panel 2A responds to concerns expressed by reviewer 2 on point 3, but it makes a good general point as it validates the approach.

Panels 4E-F, where we show that cells leave mitosis even when treated within inhibitors during mitosis, rather than before entering mitosis. This panel addresses a concern from reviewer 1 (point 3) and shows that Aurora B is not only required for the generation of an "initial burst" of checkpoint signal, but that it is in fact required to maintain the mitotic arrest, even if the requirement for Aurora B under these conditions might appear less stringent (based on our data in Figure 3A-B).

In Figure S1, panels B and C (the latter being a new panel), we address concerns from both referees 1 and 2 that kinetochore structure might be grossly affected. We show that Ndc80 and Knl1 are normally at kinetochores under conditions in which the checkpoint cannot be maintained. We have also gathered data that the Ska complex strongly stains kinetochores when Aurora B is inhibited (unpublished results from our laboratory). Because Ndc80 is required to recruit the SKA complex to kinetochores, this provides further evidence that the outer kinetochore is normally formed when Aurora B is inhibited.

Finally, we have addressed the "minor points" indicated by reviewer 2. Specifically:

We now report experimental settings for the indicated figures (whose numbering is now 3C-E and 4B) and indicate that these cells were treated with Hesp or Rev after mitotic entry and that MG132 was present.

We report information on the Mad2 antibody

We modified the discussion to clarify that there are no elements to regard Aurora B as a direct tension sensor.

AR3

We also thank AR3 for endorsing publication. To answer his/her concerns, we now report in two different instances in the text that the assumption by Yang et al. that 100 nM hesperadin completely abrogates Aurora B is undemonstrated, and furthermore unlikely when considering our data in Figure 2B. We now also point to the difference between RPE1 and HeLa cells to which the AR3 refers.

We also wish to thank AR3 for pointing out that our discussion of the Vanoosthuyse and Hardwick paper in our "pre-rebuttal" letter was incomplete. We could not find instances of the word "nocodazole' in the Vanoosthuyse and Hardwick paper, nor of additional spindle poisons. Rather, our impression is that these authors did indeed use the nda3-KM311 throughout their paper. In a sense, this does not impinge on our argument, as it is difficult to evaluate the possibility of the existence of "residual" microtubule "stubs" in these cells. Nevertheless, we agree with the reviewer

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that the Vanoosthuyse and Hardwick manuscript makes a rather compelling case on the role of Aurora B/Ark1 in the checkpoint in S. pombe. We also note that Petersen and Nurse (2003) had already made a case for a role of Aurora B in the checkpoint in S. pombe (we of course cite the paper in our manuscript). From many personal conversations with colleagues, I think it is fair to claim that the field remains unclear why it has been easier to expose a checkpoint role of Aurora B in *pombe* relative to other organisms. Many people regard it as the exception that confirms the rule. Personally, I feel inclined to think that when exceptions of such relevance occur in highly conserved machinery, they are not exceptions at all, but my feeling remains that there is unrest on the topic.

In our manuscript, we tried avoiding very detailed descriptions of the ways in which each previous experiment provided evidence in support or against a role of Aurora B in the checkpoint, specifically because several different approaches have been used. What we have tried to point out in our manuscript is that the issue of Aurora B in the checkpoint is unsettled, despite solid previous evidence in favor of a role of Aurora B ("independently of error correction").

AR3 also states that equating time to adaptation or duration of mitotic arrest to the measure of checkpoint signal strength is inaccurate. This is because other factors such as the turnover of Cdc20 and Cyclin B play important roles in dictating whether cells remain arrested or exit from mitosis after a drug-induced arrest. Our understanding is that the turnover of Cdc20, which in turn determines the basal degradation rate of Cyclin B, is set by the checkpoint, as shown by the Pines group. We have gathered similar evidence in our laboratory. On the other hand, we agree with the reviewer that we do not have accurate ways of measuring the "strength" of the checkpoint. We use duration as a surrogate marker.

We now cite the Genes Dev. 2003 Jan 1;17(1):101-14 reference, as requested by AR3.

Editors note: There are no referee reports associated with this Peer Review Process File, since the manuscript was transferred with referee reports from another journal.

2nd Editorial Decision 20 February 2011

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal. I am sorry for the delay, but in accordance with journal policy, I wanted one of the referees to evaluate the new data added. Unfortunately, this referee got delayed due to unforeseen circumstances. S/he has indicated that the manuscript should be published in the EMBO Journal.

I do have some minor comments about the statistical descriptions in figures 2, 4 & 6: In 2 & 6 you state 'sd derived from at least 50 cells' - normally we'd want exact numbers for n (and n is not independent repeats, but as long as that is made clear, this is OK). I did not see any stats description for fig 4 - did I miss this? Otherwise please add this in proof.

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